

EVALUATION OF THE WAX AND SCENT GLANDS IN THE APINAE (HYMENOPTERA: APIDAE)¹

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ABSTRACT

The wax glands of bees of the subfamily Apinae are actually specialized hypodermis which internally covers the exoskeleton in certain abdominal areas. Characteristics of glandular tissue are distinct only during the period of active secretion, and is difficult to distinguish from unspecialized hypodermis during the inactive period.

These glands are situated in sterna IV to VII in honeybees (*Apis*) and from terga IV to VII in the meliponids (*Melipona* and *Trigona*). In the bumblebees (*Bombus*), the most primitive social Apinae, they are found both in terga (IV to VII) and sterna (IV to VII), though more developed dorsally. In *Euglossa*, a unique non-social group of Apinae the wax glands are restricted to tergum VII. There is no marked difference in the histology of these glands among the species observed.

Glandular tissue, histologically similar to the wax glands, was confirmed within the mandibles of *Apis* and the meliponids. The scent glands were studied in *Bombus* workers, *Apis* workers, and meliponid queens. Scent glands were situated in the anterior part of tergum VII and showed no histological difference among species examined.

Bees of the subfamily Apinae (Michener, 1944) offer numerous interesting problems because of their remarkable social organization.

Recently, comparative studies on the evolution of Apinae bees have been promoted in the Department of General Biology of F. F. C. L. Rio Claro by my colleagues and myself in diverse aspects. The evolution of the wax and scent glands in Apinae presented here is based upon observations of their anatomy and histology.

MATERIAL AND METHODS

The following species were studied:

Euglossa cordata Linnaeus—females; *Bombus atratus* Franklin—workers; *Melipona quadrifasciata anthidioides* Lepeletier and *Melipona rufiventris* Lepeletier—drones, workers and queens; *Trigona (Scaptotrigona) postica* Latreille; *Trigona (Friseome-*

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litta silvestrii (Friese) and *Trigona (Friseomelitta) freiremaiai* Moure (M.S.)—drones, workers and queens; *Apis mellifera ligustica* Spinola drones, workers and queens.

For histological observations the material was embedded in paraffin, sectioned 10 to 15 μ in thickness and stained with Delafield's hematoxylin and eosin.

WAX GLANDS The wax glands in bees consist of specialized hypodermis which covers internally the exoskeleton of certain abdominal areas. In an adult bee the hypodermis is represented as a thin epithelium apparently syncytial, because the cell boundary is invisible. In certain areas of the body, the nuclei of such epithelia are hardly visible and the exoskeleton appears covered by a basal membrane which apparently is not cellular (Fig. 1). Structure typical to glandular tissue does not appear in the

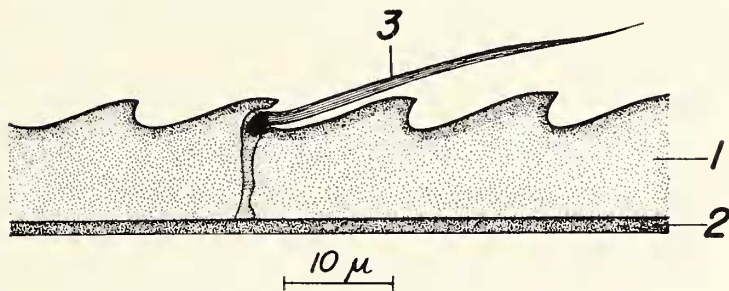


Fig. 1. Chitinous cuticle limited internally by the basal membrane: 1. Chitinous cuticle, 2. basal membrane 3. hair.

entire abdominal hypodermis but only in certain restricted areas of some sex or caste.

In the non-social bees, wax glands have not been recorded, even in those rather close to the social bees. According to von Ihering (1903), however, Moebius found nests of the genus *Euglossa* internally covered by wax or a similar substance. The histological observation of *Eu. cordata* proved the occurrence of a wax gland in this species. It is very small, and confined to the penultimate abdominal dorsal segment (Fig. 2 A).

Wheeler (1928) wrote that, the males and queens of the genus *Bombus* can produce wax. Their wax glands are situated both dorsally and ventrally in the abdomen and the glandular epithelium appears on terga IV to VII and sternae IV to VII (Fig. 2 B), though glands are more developed dorsally. This greater development is caused by the larger number of glandular cells, their greater height and greater amount of granules within them.

In *Melipona* and *Trigona* the wax glands are dorsally situated, on terga IV to VII (Fig. 2 C) as in *Bombus*. In all drone specimens examined, including even those of the most primitive species (*Trigona (Friseomelitta) silvestrii* and *Trigona (Friseomelitta) freiremaiai*), I could detect no trace of wax glands. Nevertheless, Drory (1873, 1874 and 1877) reported the wax production by males of *Melipona marginata* and *Melipona scutellaris*, which was doubted by Schwarz (1948) but reconfirmed by Kerr (1951), who observed a number of males of *Melipona marginata* with wax

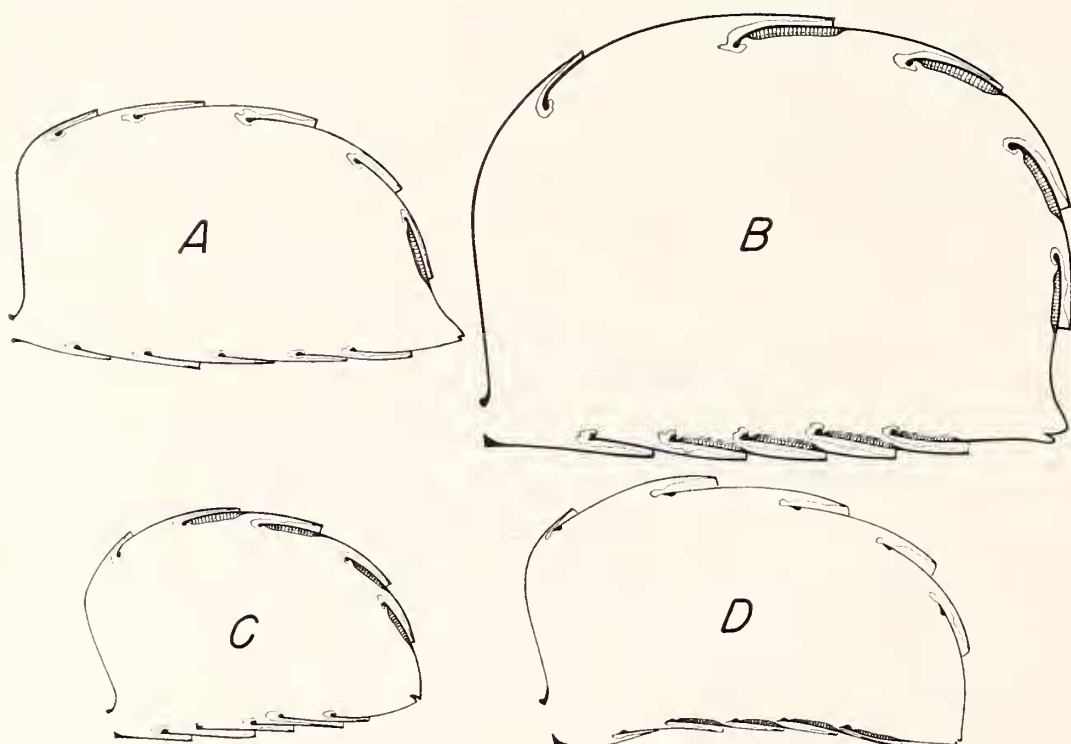


Fig. 2. Localization of wax glands: A. *Englossa cordata*; B. *Bombus atratus*; C. *Melipona quadrifasciata*; D. *Apis mellifera*.

scales on their backs. On the other hand, the queens of all species examined show well developed wax glands with the localization similar to the workers. Finally, the glandular specialization appears in *Apis* only ventrally, occupying sterna IV, V, VI, and VII of workers (Snodgrass, 1956). (Fig. 2 D.)

Since Dreyling (1903) it has repeatedly been reported that the wax glands of *Apis* suffer development and degeneration according to age throughout adult life. Confirmation of this was possible when I observed *Melipona* and *Trigona* of various ages. In the young workers immediately after emergence of *Melipona quadrifasciata anthidioides*, the hypodermis localized in terga IV,

V, VI, and VII presents no particular differentiation, shows a flat epithelium with a cell boundary hardly visible (Fig. 3 A). Corresponding to the increase of activity and age these cells become taller and the cell boundary becomes more clearly visible (Fig. 3 B). At the climax of wax production, the glandular cells are seen remarkably tall and longitudinally striated, containing numerous basophilic granules within. At this stage the empty spaces appear among cells (Fig. 3 C), representing intercellular bridges connecting the cells, as seen in a transverse section (Fig. 4).

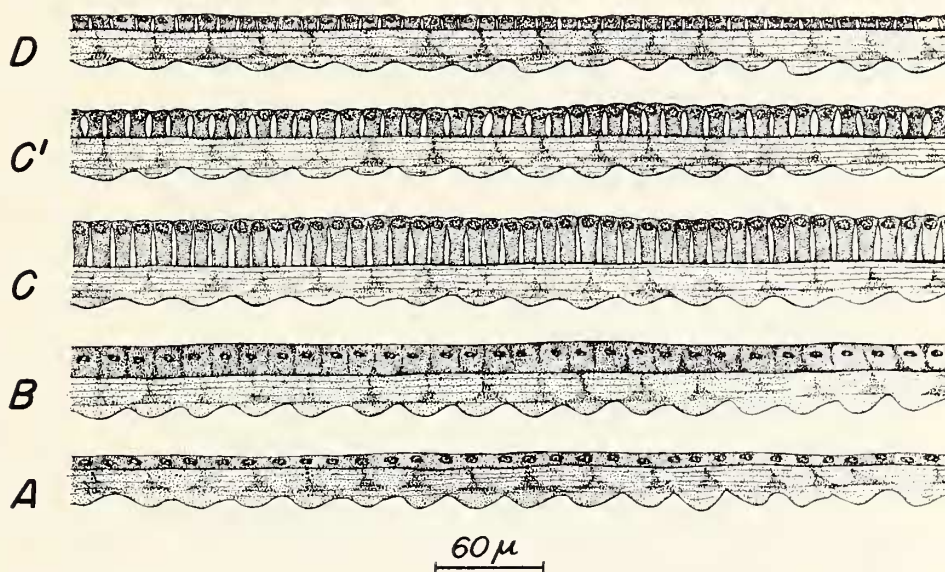


Fig. 3. Secretion cycle of wax glands: A. Undifferentiated hypodermis; B. Beginning of differentiation; C. Active period of wax secretion; C'. Beginning of degeneration; D. Degenerated glandular epithelium.

The most active period of wax secretion, that is, the period of the greatest development of the glands, in *Apis*, occurs between 12 and 18 days (Rösch, 1927). For *Melipona*, such determination has not yet been made. Kerr and Santos Neto (1953) observed the participation of the workers of *Melipona quadrifasciata* in the wax manipulation from the 12th to after the 35th day. In *Trigona (Scaptotrigona) xanthotricha* the wax production begins on the 34th day and continues to the 50th day (Hebling, Kerr, and Kerr, 1962). However, the age at which a bee presents the maximum activity of glands may vary considerably according to the needs of the hive. When the wax is urgently needed for the hive, even the foraging bees, which under normal circumstances had ceased wax production can again produce.

After the active period of wax production, the glands may suffer degeneration and the glandular epithelium becomes similar to the undifferentiated state (Fig. 3 D). In general, the regression of wax glands does not happen immediately at the end of the secretion cycle and relatively well developed glands may be found in foragers that are not too old.

The hypodermis, once differentiated into glandular epithelium during the active period of wax production, does not return to the primitive undifferentiated state in the post-active period, but shows the apparent sign of degeneration (Fig. 3 D).

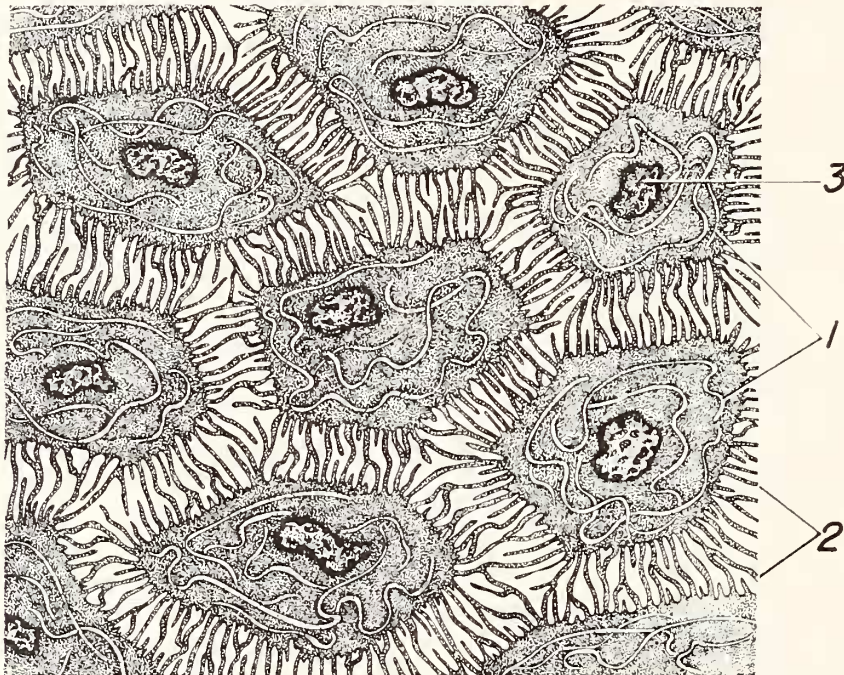


Fig. 4. Transverse section of glandular epithelium at maximum wax secretion: 1. Epithelial cells; 2. Intracellular bridges; 3. Nucleus.

During the secretion of wax it is possible to see fat cells and oenocytes closely connected to glandular cells. The fat cells on the surface of the secretory cells are flat and smaller than those found in other areas of the body (Fig. 5). Such deposition leads to the presumption that the oenocytes and fat globules may play a role in wax elaboration, probably producing lipids which enter into wax composition.

No essential histological differences were detected in wax glands of different genera and species examined. All species studied revealed a similar cycle of secretion and histology.

The secreted wax changes to the cuticular scales which cover the secretory area. How wax reaches beyond the integument

after production is still unclarified. In *Apis* the cuticle upon the wax mirrors bears a thin and semi-transparent structure. Lewke (1950) described the occurrence of canaliculi penetrating the cuticle, whereas Reiman (1952) failed to confirm the existence of such pores. He assumed that the wax may penetrate the cuticle dissolved in a special secretion which then evaporates upon reaching the external surface. This results in the condensation of wax and ultimate production of solid scales.

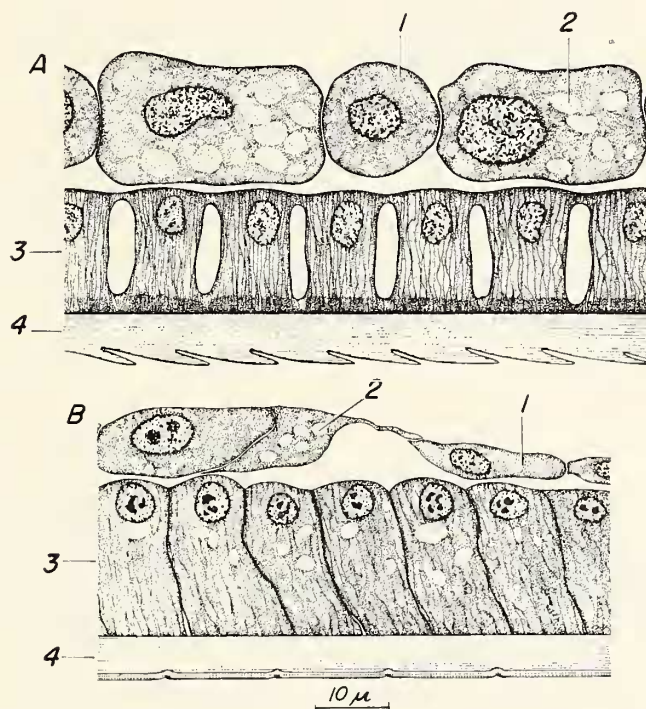


Fig. 5. Relation among oenocytes, fat cells and wax cells during the active period of secretion: A. *Melipona quadrifasciata* ♀; B. *Melipona rufiventris* ♀. 1. Oenocyte; 2. Fat cell; 3. Glandular epithelium; 4. Chitinous cuticle.

In the bees examined (except *Bombus*) no special structure in the cuticle covering the glandular area could be verified. The cuticle is transversely striated, as it is in other areas of the body. In *Bombus* the cuticle is remarkably thick, with special striations and having pores filled with an amorphous basophilic material, well stained by hematoxylin (Fig. 6).

After secretion, the wax forms the scales which are later removed by the hind legs (Fig. 7). In order to determine whether diverse depositions of glands correlate to the structure of hind legs, the relative length of each segment was measured, but no statistically significant correlation was found. The legs were

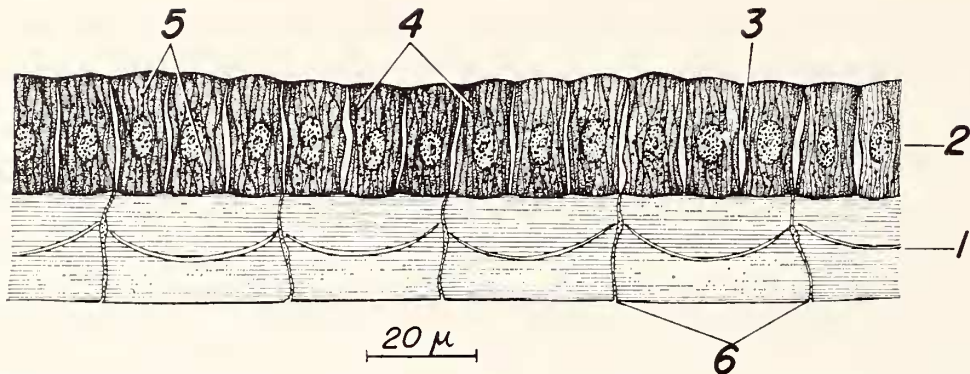


Fig. 6. Glandular wax epithelium at maximum secretion in *Bombus atratus* ♀: 1. Chitinous cuticle; 2. Glandular epithelium; 3. Intracellular space; 4. Intracellular striation; 5. Intracellular granules; 6. Intrachitinous pores.

inserted more laterally in *Apis*, *Melipona* and *Bombus* while more ventrally in *Trigona*.

WAX MANIPULATION Bees use the legs and mandibles to model wax for building and repairing the hive architecture. Orözi-Pál

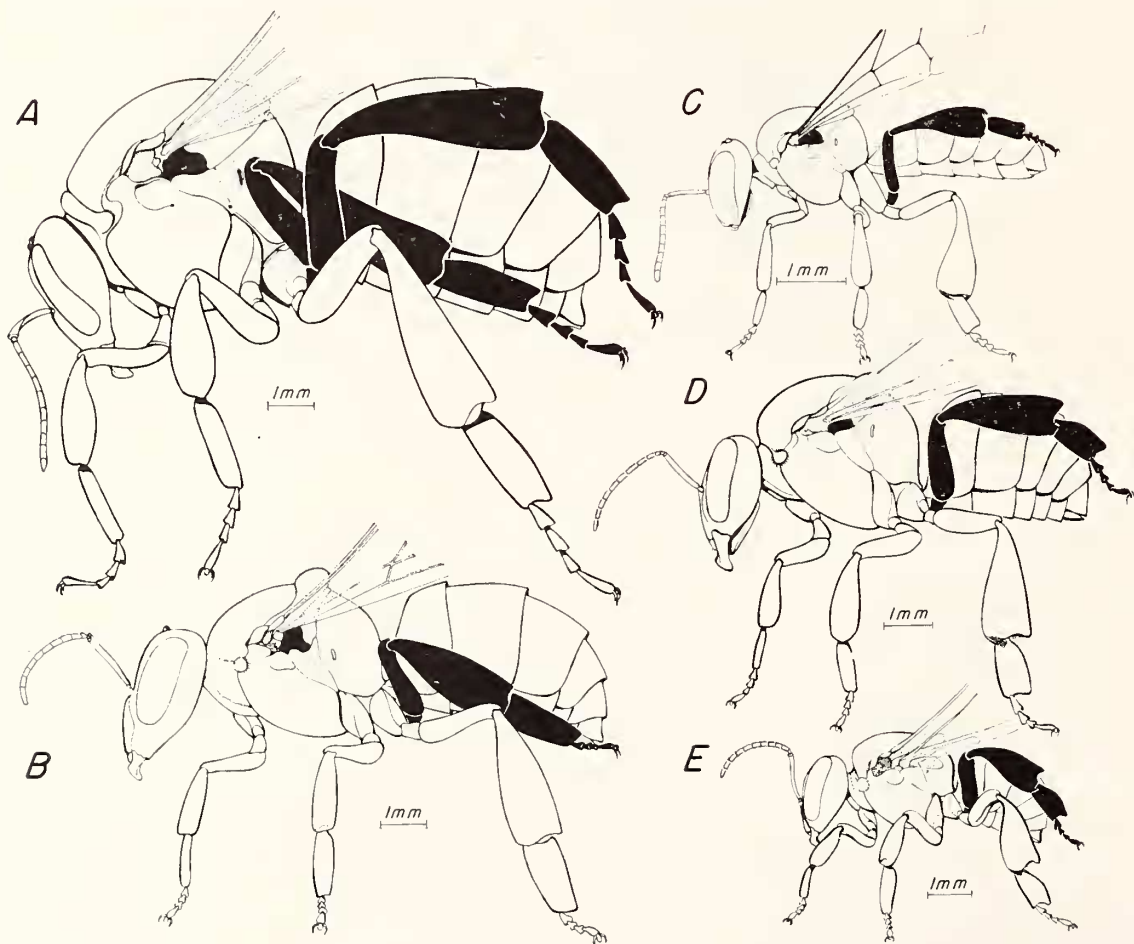


Fig. 7. Movements of hind legs for wax scale-removal: A. *Bombus*; B. *Apis*; C. *Trigona (F.) silvestrii*; D. *Melipona*; E. *Trigona (S.) postica*.

(1957) found in *Apis* that the secretion produced by the cells of mandibular glands has significance in working with wax, since the workers use the secretion to make the wax softer and easier to model. We found that the secretion of the mandibular glands of *Trigona* (*Oxytrigona*) *tataira* (Kerr and Cruz, 1961), and also *Melipona quadrifasciata* is capable of dissolving wax. In pre-

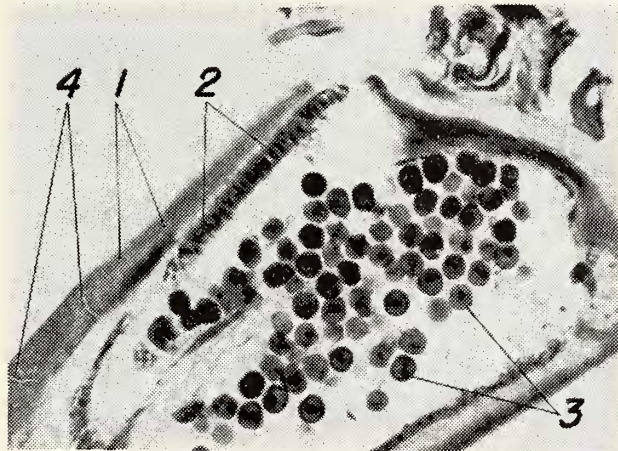


Fig. 8. Intramandibular gland of *Trigona* (*S.*) *postica* ♀: 1. Chitinous cuticle; 2. Glandular epithelium; 3. Oenocytes; 4. Intracuticular pores.

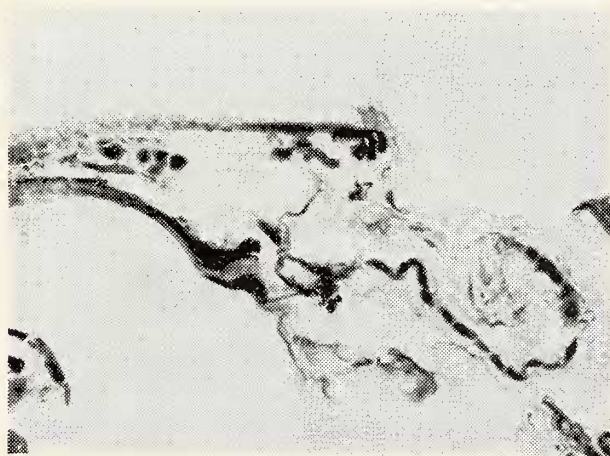


Fig. 9. Intramandibular gland of *Trigona* (*S.*) *postica* ♂.

paring histological sections of mandibles of *Melipona*, *Trigona*, and *Apis*, it was noticed that the workers of these bees have the intramandibular epithelium similar to that of the wax glands during the active period of secretion (Fig. 8). Oenocytes and fat cells within the mandibular glands also occurred. The chitinous cuticle which externally covers the mandibles has the pores filled with amorphous basophilic material, identical to that of the cuticle covering the wax glands of *Bombus*. In the queens of *Melipona* and *Trigona*, such epithelium, occurred though less

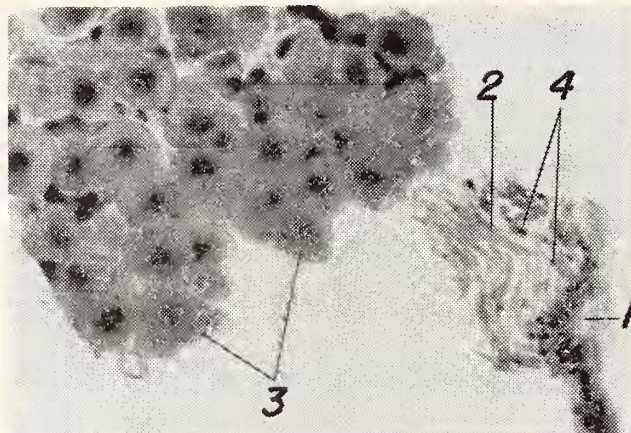


Fig. 10. Scent gland of *Melipona rufiventris* ♀: 1. Intracuticular end of excretory ductules; 2. Excretory ductule; 3. Scent cells; 4. Nuclei of ductules.

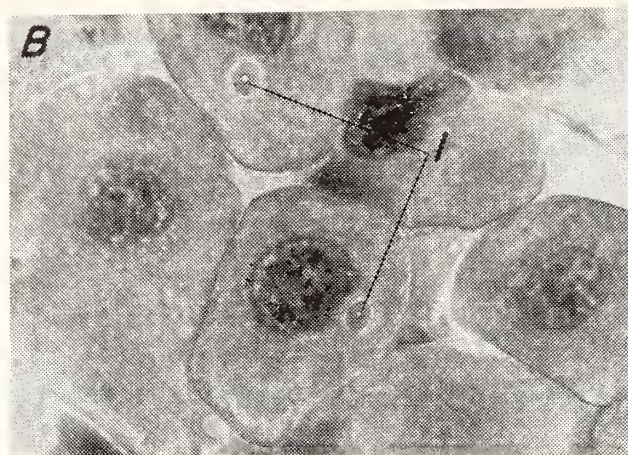
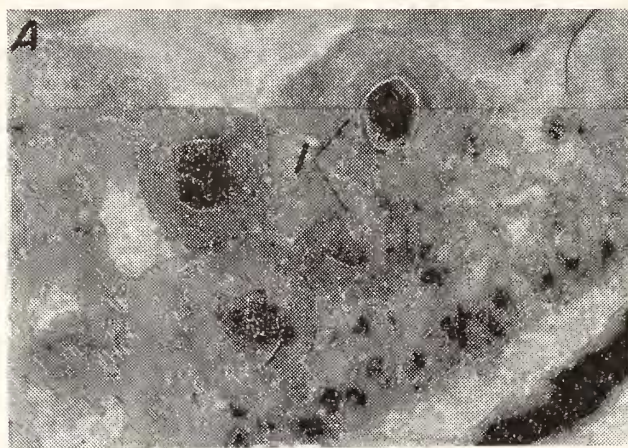


Fig. 11 (A and B). Scent gland of *Trigona postica* ♀ greatly magnified. 1. Intracellular portion of the ductule.

developed. The occurrence of glands could not be detected in drones of any species (Fig. 9).

It was assumed that glands other than mandibular glands were responsible for softening the wax. But, the finding of glands

within the mandibles similar to the wax glands suggested that these glands would be the source of the workers' wax solvent. This was tested by placing a small piece of wax on a slide. On one side of this wax there was arranged an extramandibular gland filled with secretion and on the other side an intact mandible. It was found that both glands have the capacity to soften wax since after a certain time they dissolved the surrounding wax.

SCENT GLANDS The scent gland is located in the anterior part of tergum VII in the area normally covered by tergum VI. Its structure in *Apis* was described by McIndoo (1914) and Jacobs (1924). According to them, this gland is composed of a group of cells, each provided with a delicate individual ductule, originating intracellularly at a space called an ampulla. The ductule is connected to the outside through a pore penetrating the chitinous cuticle.

Opinions of these two authors agree in admitting the absence of the scent gland in the drones of *Apis*, but disagree about its presence in the queens. McIndoo states it is present whereas Jacobs that it is absent. Since I could not find the gland in the *Apis mellifera ligustica* queen I am in accord with Jacobs.

In meliponids, the occurrence or absence of this gland in drones was not studied. It is absent in workers but well developed in queens with the location homologous to that in *Apis*. In general, no marked histological difference of the gland was observed between *Apis* and meliponids. It consisted of a group of more or less spherical cells, each provided with a chitinous ductule originating inside the cells. The ductule gives several turns within the cytoplasm around the nucleus before leaving the cell. Fig. 10. The cytoplasm around the ductule appears thicker and more stainable by hematoxylin. Fig. 11 & 12. Among the secretory cells of the scent gland can be found cells smaller in size. Each ductule is unicellular and is provided with a nucleus located laterally and immediately before its penetration to the cuticle. The presence of a scent gland was confirmed in workers of *Bombus* with no marked difference from species already mentioned.

CONCLUSION

Within the subfamily Apinae the wax gland appears even in

Euglossini, the unique non-social group but phylogenetically closely related to Bombini, which show a social organization in the most primitive state. In the latter the wax gland reaches greater development than in other species studied, and found in four segments both in dorsal and ventral sides. Whereas these glands develop only in the dorsal side in meliponids and the ventral side in *Apis*. This may be explained by secondary degeneration of either dorsal or ventral gland in these highly social groups, suggesting the primitive nature of *Bombus* in this aspect. The fact that the dorsal wax glands of *Bombus* are more developed than the ventral ones brings this genus closer to the meliponids than to *Apis*. The disappearance of the dorsal wax glands in *Apis*, and of the ventral ones in the meliponids corresponds to a specialization of these bees.

The fact that all castes of *Bombus* are able to produce wax (Wheeler, 1928) shows that the castes in this group are still not well differentiated. In the meliponids as seen above the queens retain the glands but seldom produce wax and drones of some species do not have wax glands. The maximum specialization is attained in *Apis* in which only workers possess functional glands.

The scent glands appear in workers and queens of *Bombus*, in workers of *Apis*, and in queens of meliponids. The secretion of these glands is odoriferous and in *Apis* serves to integrate each hive by the so-called colony specific odor.

In the meliponids this function is performed by the extra-mandibular gland which produces an odoriferous secretion. Hence, the disappearance of the scent gland in workers shows they took another direction in the evolution of the function concerned. The permanence of these glands in queens may be explained since this caste is always less specialized than the workers, for the queens sole role in the maintenance of the colony is as a unique reproductive agent. Studying the poison glands of the stingless bees, Kerr and de Lello (1962) also concluded that queens are more primitive than workers in this respect.

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